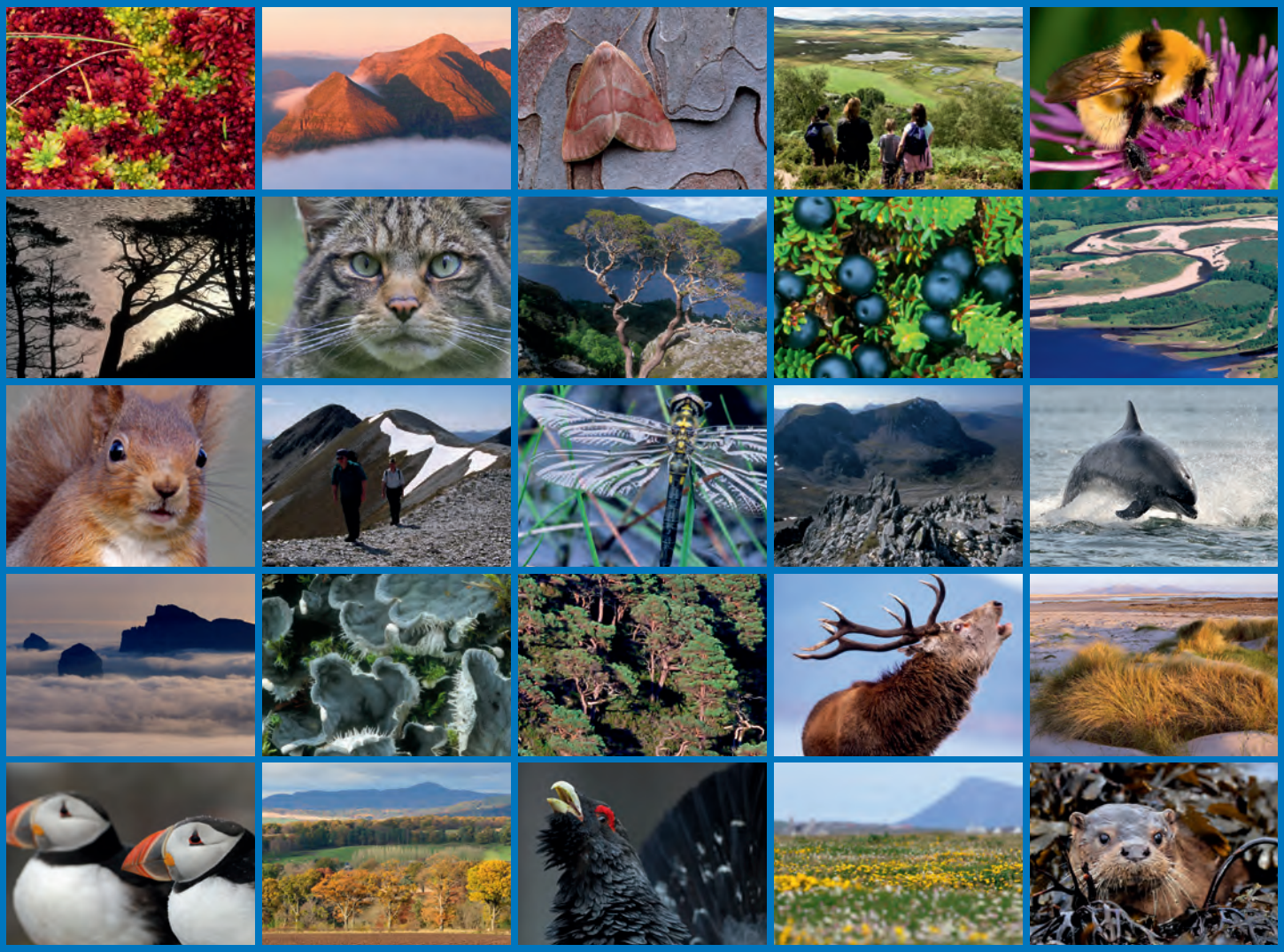
Hydrobiidae on North Uist

Scottish Natural Heritage
Commissioned Report No. 559

Hydrobiidae on North Uist



Scottish Natural Heritage
Dualchas Nàdair na h-Alba
All of nature for all of Scotland
Nàdar air fad airson Alba air fad

COMMISSIONED REPORT

Commissioned Report No. 559

Hydrobiidae on North Uist

For further information on this report please contact:

Stewart Angus
Scottish Natural Heritage
Great Glen House
INVERNESS
IV3 8NW
Telephone: 01463 725000
E-mail: stewart.angus@snh.gov.uk

This report should be quoted as:

Chevalier, M., Pye, S., Porter, J. & Chambers, S. 2014. Hydrobiidae on North Uist. *Scottish Natural Heritage Commissioned Report No. 559.*

COMMISSIONED REPORT

Summary

Hydrobiidae on North Uist

Commissioned Report No. 559
Project No: 12934
Contractor: Chevalier, M., Pye, S., Porter, J. & Chambers, S.
Year of publication: 2014

Keywords

Uists; lagoons; lochs; molluscs; snails; identification; DNA; Outer Hebrides.

Background

Mud snails (family Hydrobiidae) are small (1-4mm) benthic, aquatic gastropods that occur in a range of habitats from brackish to shallow sea water in soft substrates. They may be found living at very high densities, (up to 300,000/m^2, Linke, 1939) and therefore may contribute significantly to the energy flow and mineralisation within their habitats (Fenchel, 1972). Often Hydrobiidae species co-exist within one habitat and in abundant numbers, e.g. *Peringia ulvae, Ventrosia ventrosa* and *Hydrobia acuta neglecta.* There is uncertainty over the identification of species within the family, particularly *H. acuta neglecta* which is easily confused with other species of the Hydrobiidae. An added difficulty is that morphological characters such as shell shape, body size and tentacle pigment patterns can be affected by environmental and parasitic factors (Muus, 1963; Cherrill & James, 1987; Barnes, 2005; Wilke *et al*., 2002,). *H. acuta neglecta* is recorded in the British Isles from a small number of sites (Kerney, 1999), but many of these records cannot be verified as there are very few corresponding specimens to be found deposited in museum collections.

North Uist was chosen for this survey based on a previous Scottish Natural Heritage (SNH) survey (*Tolypella*) where the presence of *H. acuta neglecta* in both Loch an Duin and Loch an t-Sruith Mhoir was confirmed by identification of specimens, deposited in the National Museums of Scotland. These lochs are of particular importance as they are both saline lagoons that lie within the Loch nam Madadh, Special Area of Conservation. The aim of this study is to confirm the presence of *Hydrobia acuta neglecta* on North Uist using genetic analysis, and explore the use of tentacle pigmentation for identification of the species of Hydrobiidae in these sites. This report is not intended to be a guide to identification.

Main findings

- *Hydrobia acuta neglecta* was found in all three lochs sampled, identification was confirmed by genetic analysis.
- Separation of species by tentacle pigmentation was confirmed by genetic analysis.
- *H. acuta neglecta* was the most common species of Hydrobiidae sampled.
- *Peringia ulvae, Ventrosia ventrosa* and *Potamopyrgus antipodarum* were also found.

For further information on this project contact:
Stewart Angus, Scottish Natural Heritage, Great Glen House, Inverness, IV3 8NW.
Tel: 01463 725234 or stewart.angus@snh.gov.uk
For further information on the SNH Research & Technical Support Programme contact:
Knowledge & Information Unit, Scottish Natural Heritage, Great Glen House, Inverness, IV3 8NW.
Tel: 01463 725000 or research@snh.gov.uk

Table of Contents **Page**

List of Figures **Page**

List of Tables **Page**

Acknowledgements

Financial support for the survey was provided by National Museums of Scotland (NMS) and the British Trust for Conservation Volunteers Natural Talent Apprenticeship scheme. Financial support for the genetic analysis was provided by Scottish Natural Heritage (SNH). George Macdonald kindly gave permission for us to sample Lochs on North Uist Estate.

1. INTRODUCTION

The taxonomic history of the Hydrobiidae is confused because they are small, often poorly preserved, inadequately described and illustrated. *Hydrobia acuta* (Drapernaud, 1805) was originally described from Southern France, *Hydrobia neglecta* Muus, 1963 from Denmark, *Peringia ulvae* (Pennant 1777) from Flintshire (NE Wales), and *Ventrosia ventrosa* (Montagu, 1803) from the Kent coast. Wilke *et al.* (2000), using molecular techniques on specimens including topotypes, showed *Hydrobia acuta* and *Hydrobia neglecta* to have small but distinct genetic differences, and this when considered together with their geographical separation (average 4000km) "may justify considering them subspecies of the same taxon: *H. acuta acuta* and *H. acuta neglecta"*. We have followed Wilke *et al.* (2000), in applying this subspecies name. Since the first descriptions of the species, the nomenclature used has varied. We have followed Anderson (2008), except in Table 1 which keeps the names cited in previous reports.

There are published records of *Hydrobia neglecta* Muus from the Outer Hebrides (Castell & Ellis, 1964; Kerney, 1966; Waterston, 1981; Moore *et al.*, 2006). Unfortunately, few of the specimens on which the records were published, appear to have been retained. More recently specimens of Hydrobiidae were collected during the *Tolypella* survey (Scott *et al.*, 2015), in two lagoons on North Uist; Loch an Duin and Loch an t-Sruith Mhoir. It was decided to re-sample these sites and also include a third lagoon, Oban a'Chlachain to compare the distribution of different Hydrobiidae against a salinity gradient.

Morphological characteristics of *H. acuta neglecta* remain ambiguous, depending on whether samples were examined alive or preserved. Shell form and size can also be affected by environmental and parasitic factors (Wilke *et al*., 2002; Muus, 1963; Barnes, 2005). Additionally, phenotypic characters are not uniform and there is a possibility of intermediate characteristics (Wilke & Pfenniger, 2002).

Genetic analyses of the mitochondrial CO1 gene of 50 Hydrobiid specimens collected for this study were conducted at Heriot-Watt University in June 2011. The only genetic data available for *H. acuta neglecta* from British localities are from Suffolk (Wilke *et al*., 2000).

All species of Hydrobiidae collected were sequenced in order to provide comparative information. The combination of morphological characters recorded from living samples, salinity and molecular analysis results provided information on the presence of the Hydrobiidae from North Uist. The value of using head and tentacle pigment pattern to separate hydrobiid taxa including *H. acuta neglecta* will be considered.

1.1 Previous surveys

There have been a number of surveys of the biota of the lagoons in North Uist but few specimens have been retained for verification.

Nicol's publication in 1936 was the first looking at the biota of the lagoons in North Uist, but *Hydrobia neglecta* was only described in 1963. The first published record of specimens recorded from Uist was in 1964 (Castell & Ellis, 1964), collected by Tom Warwick in the same year.

Table 1. Hydrobiidae reported by previous surveys of the lochs covered in this report. The nomenclature used by authors of the previous surveys have been retained

	Loch		
Report	Loch an Duin	Loch an t-Sruith Mhoir	Oban a'Chlachain
Nicol, 1936 No specimens found in collections	*Peringia ulvae* (x2 varieties), *Hydrobia ventrosa*	*Peringia ulvae* (x2 varieties), *Hydrobia ventrosa*, *Potamopyrgus antipodarum*	No Hydrobiid species mentioned (*P.ulvae* Clachan sound, *P.ulvae* & *H. ventrosa* in Oban Irpeig)
Kerney, 1966 No specimens found in collections		*Hydrobia neglecta* Coll T Warwick 1964 [Loch an Strumore]	
Smith, 1978 No specimens found in collections	N/A	N/A	Nil
Dipper & Mitchell 1980 No specimens found in collections	*Hydrobia ulvae, Hydrobia ventrosa, Potamopyrgus jenkinsi*	N/A	Nil
Smith, 1987 No specimens found in collections	NA		*Hydrobia ulvae, Hydrobia ventrosa*
Moore *et al.*, 2006 No specimens found in collections	*Hydrobia neglecta, Potamopyrgus antipodarum*	N/A	N/A

It is also worth mentioning that the conditions in Oban a'Chlachain may have changed since 2006, as a result of the rebuilding of the causeway and its culverts (where the sea enters the loch) after winter storms the previous year. Changes in the size of the water ingress and its orientation would affect the long term conditions in the loch. It is not known how the seawater ingress has changed, nor how the loch may have been affected.

2. METHODS

2.1 Survey

The survey was carried out between the 25th and the 28th of April 2011, by a British Trust for Conservation Volunteer (BTCV) natural talent molluscan apprentice, Melissa Chevalier and the curator of molluscs at National Museums of Scotland, Sankurie Pye. The survey base was near Oban a'Chlachain, North Uist. All surveying was shore based.

2.2 Site selection

Sites were selected close to those of a previous survey (Scott *et al.,* 2015), where hydrobiids had been found. However, sampling was restricted to locations accessible from the shore and to those sites that could be reached within the project time constraints and tidal conditions. The position of each site was recorded using a GPS (Garmin Etrex), WGS84 Datum.

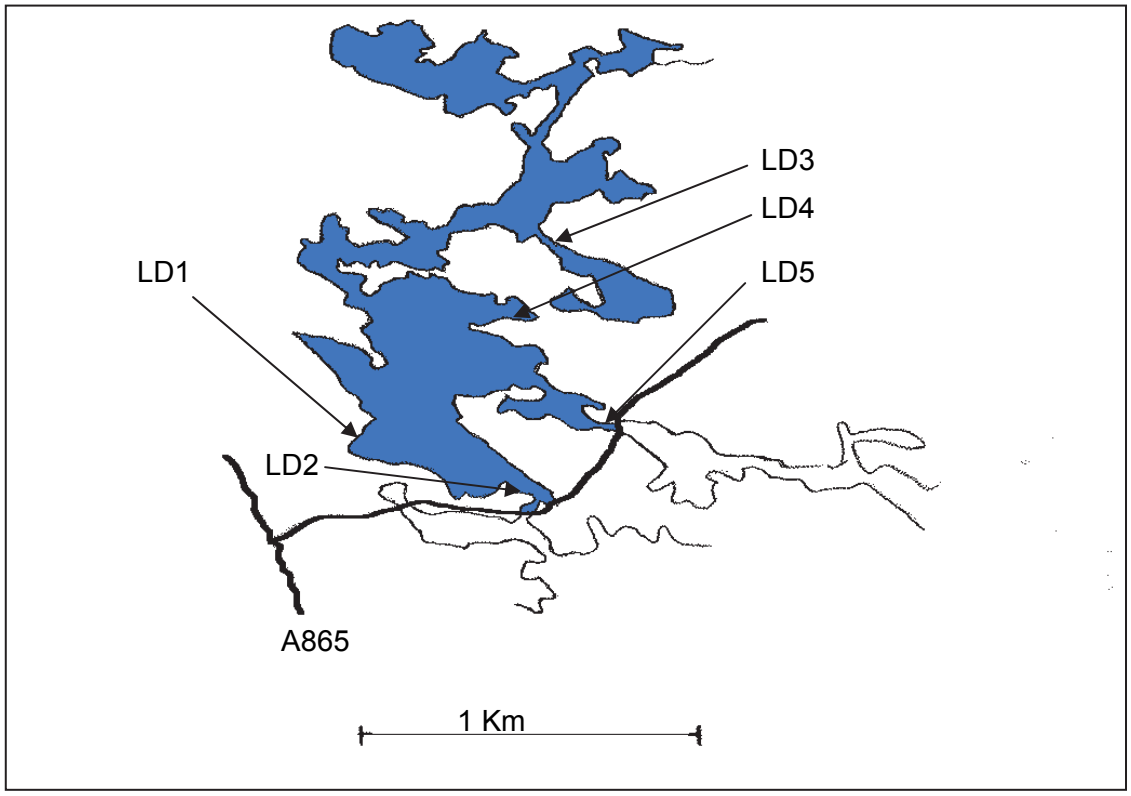


Figure 1. Map of Loch an Duin showing survey sites

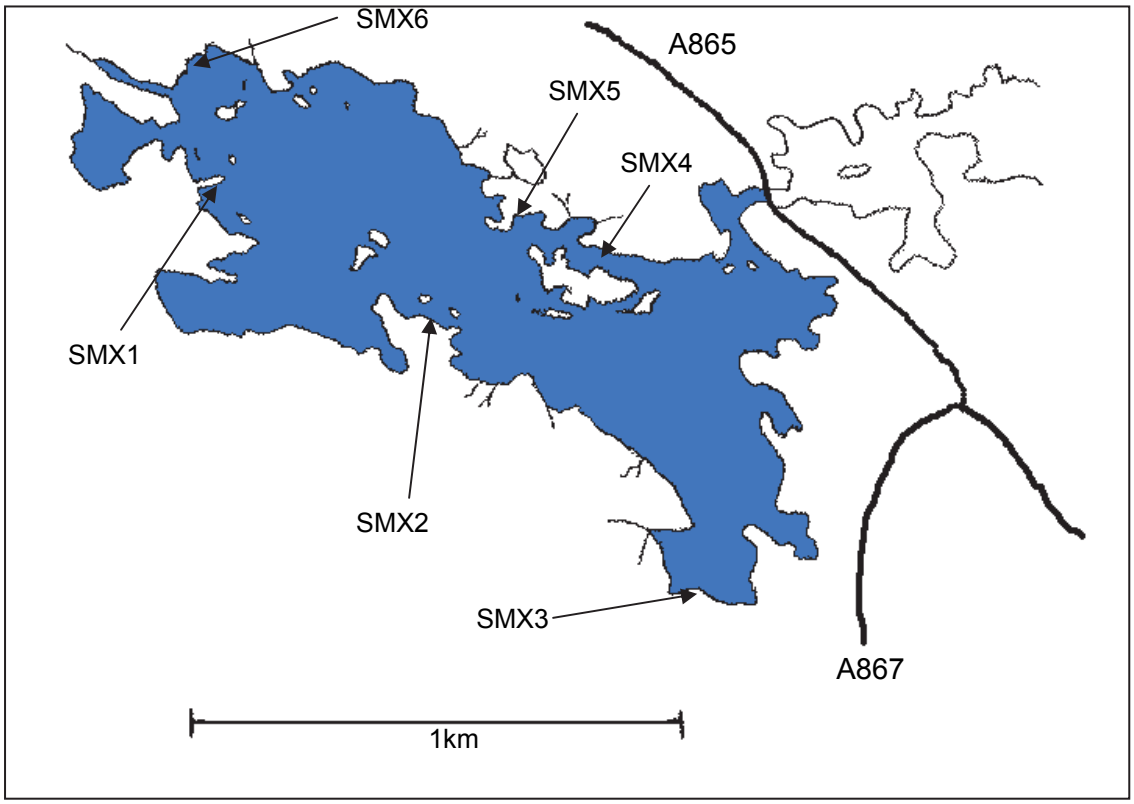


Figure 2. Map of Loch an t-Sruith Mhoir showing survey sites

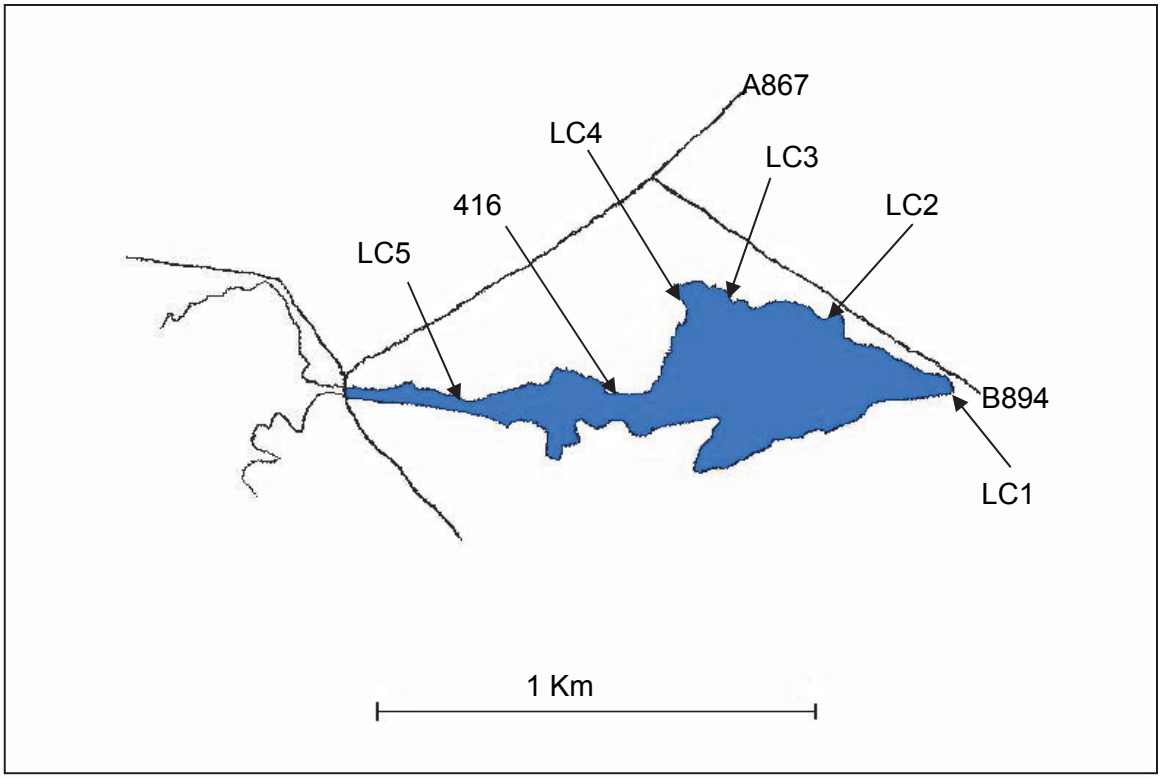


Figure 3. Map of Oban a'Chlachain, showing survey sites

2.3 Sampling techniques

Sampling was carried out over the course of three days and the identification of live specimens was completed on the day of collection. Samples were collected at low tide. Collection was made using a 1mm mesh sample net, the mouth of which was approximately 625cm^2. Mud snails were collected by a 4 stroke sweep within the upper 30 cms of the area including weed, rock and mud. Samples were then placed in 250ml plastic containers. A Wild M7A binocular microscope was used to sort the live animals by head and tentacle pigment pattern. A maximum of 20 specimens were sorted per site (sub-sampled randomly from larger samples). Illustrations (refer to Figures 4 & 5) were made of each head pigmentation type, and the specimens were then preserved in 100% ethanol for molecular extraction. All shells selected for molecular analysis were lightly pierced to allow the ethanol to penetrate the snail tissue. All material not required for molecular analysis was retained in pure ethanol and deposited at the National Museums of Scotland; Accession number NMS.Z:2011.71.x Information on specimens used for genetic analysis will eventually be submitted to GenBank.

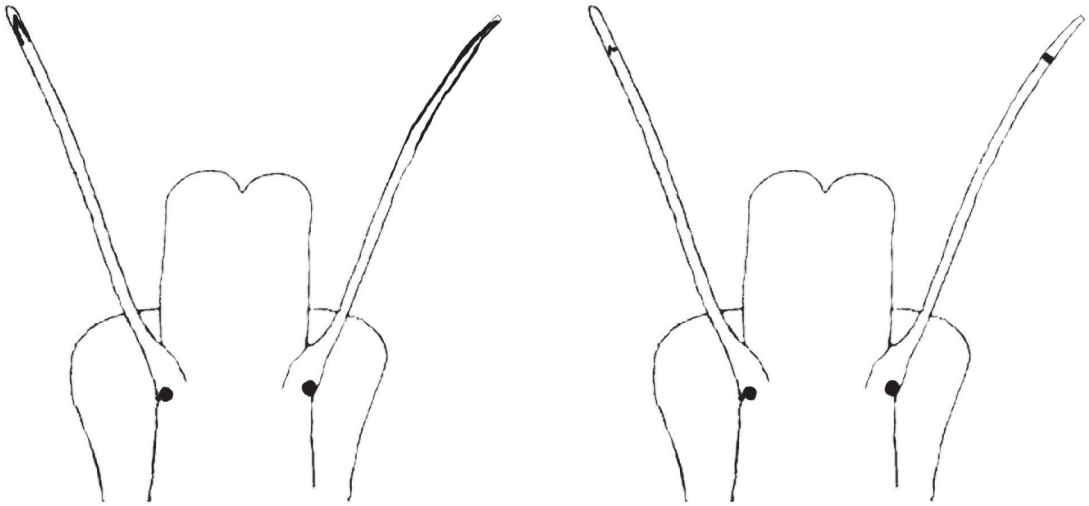
2.4 Genetic techniques

The genetic sequencing of approximately 50 specimens from 15 populations was conducted at Heriot- Watt University. DNA was extracted using the Qiagen DNEasy kit. The primers to amplify a 710 bp fragment of the CO1 gene were those of Folmer *et al.* (1994). Ready to go PCR beads (GE Healthcare) were used for the individual PCR reactions. The thermocycling protocol outlined in Wilke *et al.* (2000) was employed. Following the PCR, all amplification products were visualised on a 1% agarose gel with a 100bp DNA ladder (Promega). Gels were stained with ethidium bromide and visualised under UV illumination. PCR products from those bands on the gel identified as being pure products were then cleaned up using an Invitrogen PCR miniprep clean up kit with the High cut off buffer option. The cleaned up samples were then quantified using the Biophotometer (Eppendorf) and sequence reactions were set up using 20ng of the purified DNA. Samples were sent to the Genepool sequencing facility, Edinburgh for Sanger sequencing. The raw sequence files generated by the Genepool were edited in the Sequencher software package (Genecodes). Primer sequence was trimmed off, forward and reverse sequences were aligned, and a consensus sequence was created for each individual from the two sequences. Where two sequences could not be aligned or where sequence data were deemed to be of poor quality, they were discarded from the analysis. Sequence identities of the good quality data were checked using the BLASTn algorithm on Genbank. All the sequences had 99% matches to Genbank sequences from the *Hydrobia* group, giving a good indication that sequences were bona fide and not contaminated. Sequences were then imported in nexus format into MacClade software. Pairwise alignment was used to align the sequences and then the sequences were trimmed to the same length. The alignment file was saved in nexus format and imported into MEGA software. Model testing was performed to determine the best model of evolution to be used in the phylogenetic reconstruction. For this dataset the Bayesian Information Criterion determined that a HKY + G model was most appropriate (BIC=4821.527). Phylogenetic reconstruction was performed using this model and 500 bootstraps were performed to assess confidence of the topology. Maximum likelihood analysis was the phylogeny reconstruction method employed. Sequences from Genbank of representative species were also used in the phylogenetic reconstruction to provide benchmarks of identity.

3. RESULTS

3.1 Identification and distribution

P. ulvae and *V. ventrosa* and *H. acuta neglecta* from the three localities could be separated in living samples using tentacle pigmentation patterns. *P. ulvae* had transverse bands, *V. ventrosa* had clear or grey tentacles and *H. acuta neglecta* generally had pigmented cones (better described as an inverted V shape). This character separation was confirmed by genetic analyses and by looking at the penis morphology of a few specimens chosen at random. Penis morphology of specimens with intermediate characteristics was examined, for example some *P. ulvae* specimens had one tentacle with a small cone like pigment pattern. Head pigmentation was far more variable and therefore a less valuable character for identification. *P. antipodarum* tentacle pigmentation was not recorded as this species was never confused with the other species.



Hydrobia acuta neglecta
Two pigmented cone types;
Left; short cone, right; long cone

Peringia ulvae
Two pigmented band types
Left; uneven band, right; straight band

Figure 4. Tentacle banding of Hydrobia acuta neglecta and Peringia ulvae. The thickness and the location of the pigmentation along the tentacle are variable.

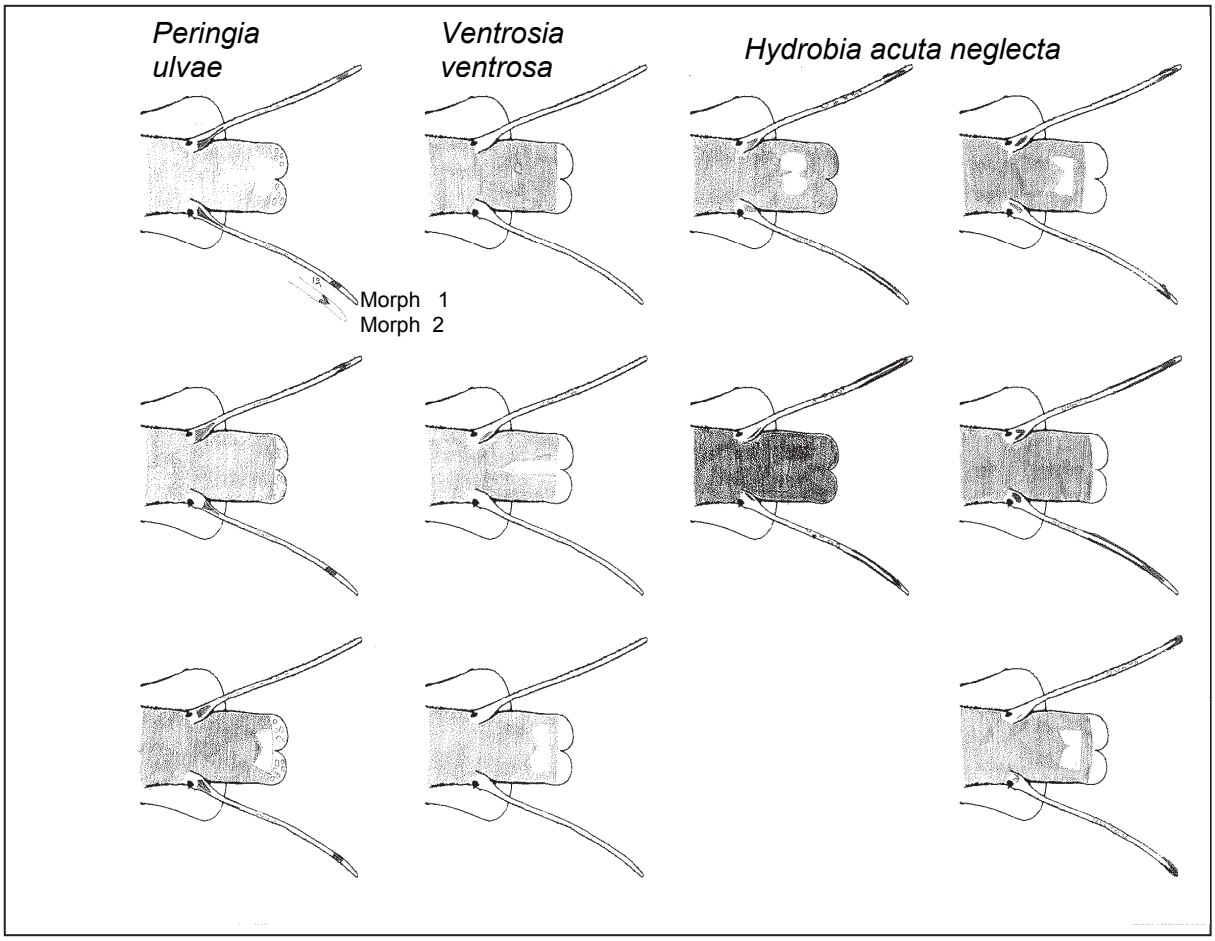


Figure 5. Sample head pigmentation patterns

Table 2. Hydrobiidae identified in this survey

Loch		
Loch an Duin	Loch an t-Sruith Mhoir	Oban a'Chlachain
Peringia ulvae		
Hydrobia acuta neglecta	*Hydrobia acuta neglecta*	*Hydrobia acuta neglecta*
Potamopyrgus antipodarum	*Potamopyrgus antipodarum*	
Ventrosia ventrosa	*Ventrosia ventrosa*	

Hydrobiids were present in the highest numbers (100s per sample) from Loch an Struth Mor and the lowest from Oban a'Chlachain (0-21 individuals per sample).

The maximum likelihood tree (Figure 6) shows three distinct monophyletic groups with high bootstrap support, and confirms the three species of Hydrobiidae found on North Uist. In Loch an Duin; *Peringia ulvae* (Pennant, T., 1777*) Ventrosia ventrosa* (Montagu, G., 1803), *Hydrobia acuta neglecta* Muus 1963. *Potamopyrgus antipodarum* (Gray, 1843) was also identified from the samples but despite repeated attempts including using different annealing temperatures DNA could not be extracted from these individuals from any location. Loch an Duin had all four species of Hydrobiidae but Loch an t-Sruith Mhoir did not include *Peringia ulvae.* In Oban a'Chlachain the only hydrobiid present in the samples was *H. acuta neglecta. H. acuta neglecta* was the most frequent species collected, as it was present in all three lochs. *Hydrobia acuta neglecta* collected from North Uist never aligned with *Hydrobia acuta*

acuta results on Genbank, confirming the separation between *H acuta acuta* and *H. acuta neglecta.*

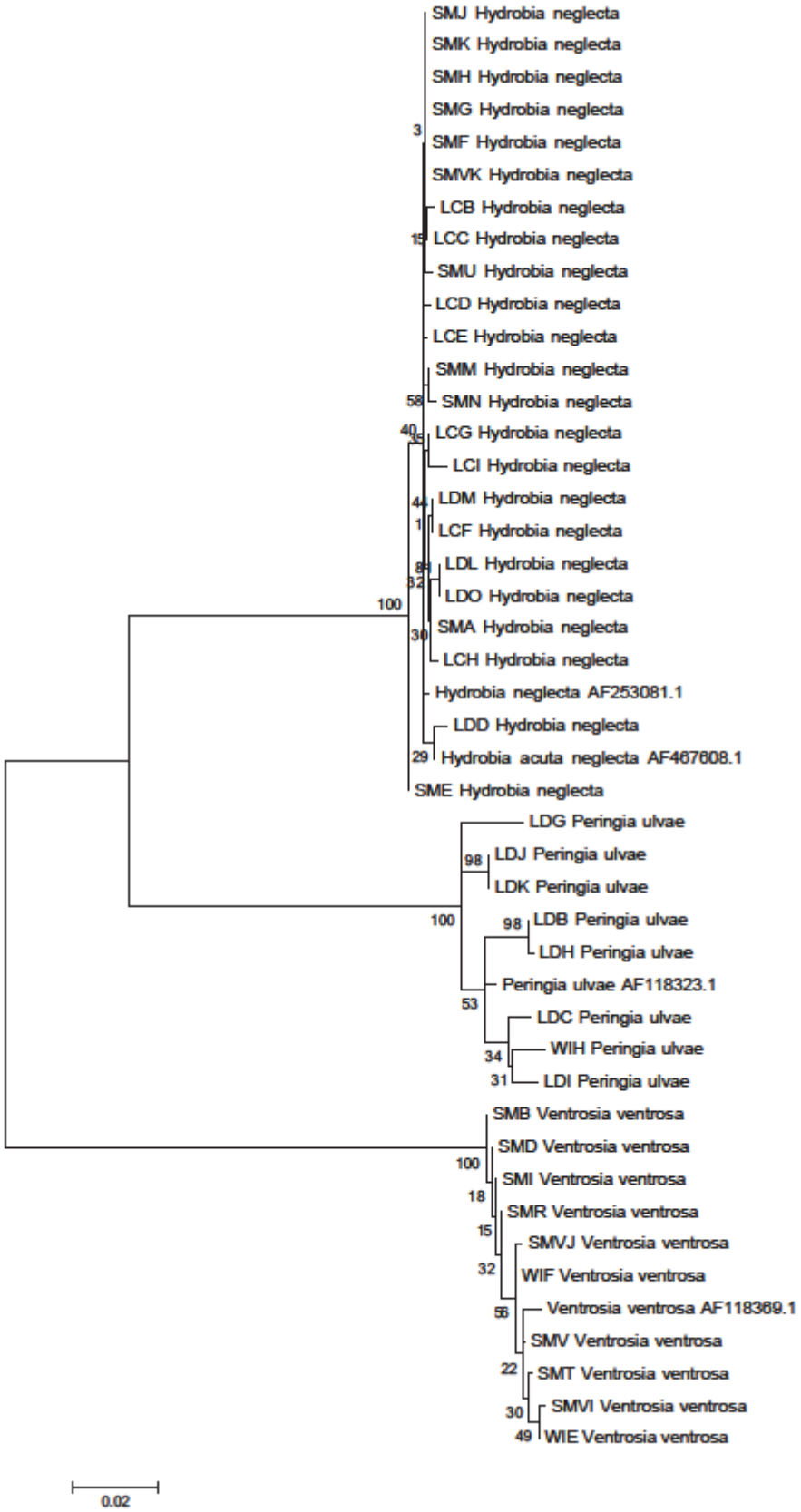


Figure 6. Hydrobia maximum likelihood tree constructed from North Uist specimens

4. DISCUSSION

4.1 Value of pigment patterns in identification

Bishop (1976) reported that *H. acuta neglecta* was characterised by the presence of pigmented cones on the tentacles. According to Falniowski (1986) *V. ventrosa* had longitudinal bands or no tentacle pigmentation and *P. ulvae* had transverse bands. These patterns were present in the North Uist samples but the cone pattern on *H. acuta neglecta* looked more like inverted Vs. There were also animals with intermediate characteristics. Falniowksi states that there is so much variation that this is not a simple 'mechanical' diagnostic feature and that other characteristics may also need to be considered. In some samples from North Uist it was difficult to separate short cone and transverse band tentacle pigment patterns (Figure 4). The length of the cone pigment pattern in *H. acuta neglecta* also varied considerably. Although there is variation, tentacle pigmentation appears to be the first character to separate species of Hydrobiidae found in the lochs in North Uist.

No link between head pigmentation and species was observed.

4.2 Phylogenetic analysis

The phylogenetic analysis shows that the sequences of Hydrobiid snails from the three lochs fall into three well supported clades. Each clade represents a different species of Hydrobiidae; *Peringia ulvae*, *Hydrobia acuta neglecta* and *Ventrosia ventrosa*. There is very little substructure within the clades. There are two explanations for this observation. Firstly it is possible that geneflow is occurring between the individual lochs that were sampled and so the populations within each species are not isolated from each other. An alternative hypothesis is that COI marker does not provide sufficient resolution to distinguish differences at the sub-species level. This issue could be addressed by trying out markers from other regions of the genome or to assess finer scale patterns within species, it may be necessary to use more variable markers such as microsatellite regions of DNA.

A caveat of using GenBank to confirm species identity is that as with all published data it is assumed that deposited Genbank data are accurate and that correct species names have been applied to the sequences that are returned as similar sequences to our own. In this study there is no reason to doubt the accuracy of the Genbank data as there were no issues in aligning the sequences generated from different laboratories.

4.3 Salinity effects on distribution

Muus (1963) suggested that there was a relationship between salinity and mud snail species distribution in Denmark, with *V. ventrosa* dominant at the lowest salinities and *P. ulvae* dominant at highest salinities but this pattern does not seem to be replicated in Britain. Barnes (1991) pointed out that levels of salinity, tidelessness and shelter in Kattegat, Denmark run parallel to each other. He concluded that the perceived connection between species of Hydrobiidae and salinity may be more about their habitat preferences. There have been many studies of the salinity ranges/preferences of the three species and salinity limits that are reported vary (Fenchel, 1975; Fretter and Graham, 1988; Barnes, 1991, 2005). There is, however, general agreement that *H. acuta neglecta* is a brackish water obligate; Barnes' 1991 report of an intertidal population of *H. acuta neglecta* in Finistère France has not yet been verified; Wilke *et al.* (2000) sequenced Hydrobiids from Barnes' Finistiere sample site but the specimens collected and sequenced were *Hydrobia glyca (*Servain, 1880*)*.

It would be expect that the Hydrobiidae species habitat distribution on North Uist to follow the distribution of other UK records, where all the species studied may be found to co-exist in the same salinity and habitat (Barnes, 1991; 2005). Fenchel (1975) suggested that water

turbulence was an important factor in determining distribution since *H. acuta neglecta* and *V. ventrosa* were only found in sheltered areas whereas *P. ulvae* was more tolerant to wave action. Further information about salinity preferences of the species on North Uist could be gleaned if the salinity of the lagoons were to be measured longer term.

4.4 Habitats

According to Barnes (1999), *P. ulvae* is rare in landlocked lagoons due to the difficulties of larval dispersal and recruitment within a lagoon compared to direct development of *V. ventrosa* and *H. acuta neglecta*. Unfortunately the lagoon definition used by Barnes (1989) is extremely narrow: "All British lagoons are isolated behind barriers of shingle". This could certainly explain the rarity of *P. ulvae* in his work, but not necessarily in the Loch nam Madadh sample sites, which by Barnes' definition would not be called lagoons. The narrow saltwater inflows of the Loch nam Madadh sites would presumably still limit recruitment of *P. ulvae* from the sea, but not quite as effectively as a shingle barrier.

Barnes (2005) noted that episodes of colonisation/extinction/ and recolonisation by hydrobids are common consequently "it may not always be possible to understand current distribution patterns on the basis of ecological interactions at any particular point in time".

5. CONCLUSIONS

Hydrobia acuta neglecta is present in all the lagoons sampled in North Uist and in greater abundance than had been anticipated. This species has been recorded at few sites in Britain, making the populations on Uist significant. As a brackish water obligate species that is not widely distributed, *Hydrobia acuta neglecta* populations are vulnerable to habitat changes that lead to changes in salinity e. g. sea-level rise.

If any species of Hydrobiidae were to be used as an indicator of lagoon status, confident identification of species would be necessary for accurate recording. Samples should be identified live, some of which are then retained and suitably preserved for confirmation by additional anatomical/genetic work.

6. REFERENCES

Anderson, R. 2008. Annotated list of the non-marine Mollusca of Britain and Ireland. Conchological Society of Great Britain. ISSN 1778-3491.

Barnes, R.S.K. 1989. The Coastal Lagoons of Britain: An Overview and Conservation Appraisal. *Biological Conservation,* **49**: *295-313.*

Barnes, R.S.K. 1991. On the distribution of the Northwest European species of the gastropod *Hydrobia*, with particular reference to *H. neglecta. Journal of Conchology,* **34**: 59-62.

Barnes, R.S.K. 1999. What determines the Distribution of coastal Hydrobiid mudsnails within North-Western Europe. *Marine Ecology*, **20** (2): 97-110.

Barnes, R.S.K. 2005. Interspecific competition and rarity in mudsnails: feeding interactions between and within *Hydrobia acuata neglecta* and sympatric *Hydrobia* species. *Aquatic Conservation: Marine & Freshwater Ecosystems*, **15**: 485-493.

Bishop, M. J. 1976. *Hydrobia neglecta* Muus in the British Isles. *Journal of Molluscan Studies,* **42**: 319-326.

Castell, C.P. & Ellis, A.E. 1964. [specimens exhibited] *Journal of Conchology*, **25**: 314.

Cherill, A. J. & James, R. 1987. Character displacement in *Hydrobia*. *Oecologia,* **71**: 618-623.

Dipper, F. & Mitchell, R. 1980. Sublittoral survey of selected marine and brackish water ecosystems of the Uists, Outer Hebrides. *Nature Conservancy Council, CSD Report No. 275.*

Falniowski, A. 1986. Pigmentation in *Hydrobia ulvae* (Pennant, 1777) amd *H. ventrosa* (Montagu, 1803) (Hydrobiidae, Prosobranchia) from the Polish Baltic. *Acta Hydrobiologica* **28**: 443-449.

Fenchel, T. 1972. Aspects of decomposer food chains in marine benthos. *Verhandlungen der Deutschn Zoologischen Gessellschaft*, **65**: 14-22.

Fenchel, T. 1975. Factors determining the distribution patterns of Mud snails (Hydrobiidae). *Oecologia*, **20**: 1-17.

Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology,* **3**: 294-299.

Fretter, V. and Graham, A. 1988. Molluscs: Prosobranch and Pyramidellid Gastropods. *The Linnaean Society of London and Brackish – Water Sciences Association*. No.2.

Kerney, M.1966. Recorder's report: non-marine Mollusca. *Journal of Conchology,* **26** (1): 77-78.

Kerney, M.1999. *Atlas of the land and freshwater molluscs of Britain and Ireland*. pp. 31-36. Colchester. (Harley).

Linke, O. 1939. Die Biota des Jadenbusenwattes. *Helgolander Wissenschaftliche Meeresuntersuchungen,* **3**: 201-348.

Moore, C.G., Saunders, G., Mair, J.M. & Lyndon, A. R. 2006. The establishment of site condition monitoring of the saline lagoon, Loch an Duin, North Uist Site of Special Scientific Interest. *Scottish Natural Heritage Commissioned Report No. 150 (ROAME No. F02AA409).*

Muus, B.J. 1963. Some Danish Hydrobiidae with the description of a new species, *Hydrobia neglecta*. *Proceedings Malacological Society of London,* **35**: 131-138.

Nicol, E.A.T. 1936. The brackish water lochs of North Uist. *Proceedings of the Royal Society of Edinburgh*, **56**: 169-195.

Scott, S., Darwell, A. & Stewart, N. 2015. Bird's nest stonewort *Tolypella nidifica* in Loch an t-Sruith Mhoir and Loch an Duin, North Uist. *Scottish Natural Heritage Commissioned Report No. 461.*

Smith, S.M. 1978. Mollusca of rocky shores: North Uist, Benbecula and South Uist, Outer Hebrides. (Contractor: S.M. Smith, Edinburgh.) *Nature Conservancy Council, CSD Report No. 210.*

Smith, S.M. 1987. Obs in north-west Scotland and the Hebrides. (Contractor: S.M. Smith, Edinburgh.) *Nature Conservancy Council, CSD Report No. 801.*

Waterston, A.R. 1981. Non-marine invertebrate fauna of the Outer Hebrides. *Proceedings of The Royal Society of Edinburgh.* Section B. Part 7. Mollusca and Bryozoa, 314-321.

Wilke, T., Rolán, E. & Davis, G.M. 2000. The mudsnail genus *Hydrobia* s.s. in the northern Atlantic and western Mediterranean: a phylogenetic hypothesis. *Marine Biology,* **137**: 827-833.

Wilke, T. & Pfenniger, M. 2002. Separating historic events from recurrent processes in cryptic species: phylogeography of mud snails (*Hydrobia* spp.) *Molecular Ecology,* **11**: 1439-1451.

Wilke, T., Pfenniger, M. & Davis, G.M. 2002. Anatomical variation in cryptic mudsnail species: Statistical discrimination and evolutionary significance. *Proceedings of the Academy of Natural Sciences of Philadelphia,* **152**: 45-66.

APPENDIX

Loch an Duin	Low tide all sites	all hydrobids counted in sites for this loch				
Date	**Station**	**Grid ref**	**Salinity (ppt)**	**Notes**	**Photo**	**Time**
27/04/2011	1 (418)	NF88819 73487	20	near pine forest	LD 1 site (3 photos), LD 1 weed (1 photo)	10:15
27/04/2011	2 (419)	NF89266 73356	20		LD 2 site (2 photos), LD 2 weed (1 photo)	10:45
27/04/2011	3 (420)	NF89374 74092	15	narrow bit of loch	LD 3 site (2 photos), LD 3 weed (1 photo)	11:30
27/04/2011	4(422)	NF89248 73835	20	very little suitable weed	LD 4 site (2 photos), LD 4 weed (3 photos)	12:05
27/04/2011	5(423)	NF89578 73535	20	next to outflow	LD 5 site, inlet, weed, Mel (5 photos)	12:30
					LD general and intlets (5 photos)	

Oban a' Chlachain						
Date	**Station**	**Grid ref**	**Salinity (ppt)**	**Notes**	**Photo**	**Time**
26/04/2011	1 (411)	NF82428 63973	24	far end of Loch (E)	LC 1 site (2 photos)	10:00
26/04/2011	2 (412)	NF82151 64118	23	far end of loch nearer main road	LC 2 site (3 photos) LC 2 Weed (2 photos)	10:20
26/04/2011	3 (413)	NF81920 64199	24	wee island past seepage	LC 3 site (3 photos) LC 3 weed (1 photo)	10:30
26/04/2011	414	NF81812 64197	21	no sample, but next to biggest inflow	LC 414 (2 photos)	NA
26/04/2011	4 (415)	NF81655 63960	24	rissoids and bivalves	LC 4 site (2 photos) LC 4 weed (1 photo)	11:05
26/04/2011	416	NF81335 63937	24	no sample	LC 416 (1 photo)	NA
26/04/2011	5 (417)	NF81141 63968	25	only 10 specimens in sample	LC 5 site (2 photos)	11:30
					LC general and inlets (4 photos)	

Loch an t-Sruith Mhoir						
Date	**Station**	**Grid ref**	**Salinity (ppt)**	**Notes**	**Photo**	**Time**
25/04/2011	SM X4	NF90283 69512		on mainland shore opposite island (middle of)	SM X4 site (2 photo) SM X4weed (2photos) SM X4 recording (1 photo)	08:35
25/04/2011	SM X5	NF90115 69570		steep cliff, sticky out bit of land	SM X5 site (3 photos) SM X5 weed (2photo)	09:00
25/04/2011	SM X6	NF89457 69895		Tiny island joined to mainland by ? Old wall of stones near	SM X6 site (2 photos) SM X6 weed (2photo) SM	09:40

			FW inlet	X6 sampling (2 photos)	
25/04/2011	SM X1	NF89515 69654	End of spit sticking out	SM X1 site (2 photos) SM X1 weed (2photo)	10:10
25/04/2011	SM X2	NF89928 69373	Big bulge of land facing north 35 swans! 2 small islands in front	SM X2 site (3 photos) SM X2 weed (2photo)	11:05
25/04/2011	SM X3	NF90470 68818	small cairn? On water edge. Not far from old road	SM X3 site (3 photos) SM X3 weed (1photo)	11:35
				SM inflows, swans, snacktime & flying (13 photos)	

www.snh.gov.uk

ISBN: 978-1-85397-937-8

Policy and Advice Directorate, Great Glen House,
Leachkin Road, Inverness IV3 8NW
T: 01463 725000

You can download a copy of this publication from the SNH website.

recycle

Scottish Natural Heritage
Dualchas Nàdair na h-Alba
All of nature for all of Scotland
Nàdar air fad airson Alba air fad